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Pattern and Determinants of Antimicrobial Resistance in Uropathogenic Extended Spectrum β-lactamase Producing *Escherichia coli*

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This study aims to determine resistance pattern and the prevalence of extended-spectrum beta-lactamase (ESBL) among 250 *Escherichia coli* in Riyadh and to determine the antimicrobial resistance determinants for ESBL positive *E. coli* (ESBL-EC) which collected from urine during 2012. MICs were determined. Phenotypic and genotypic screening of ESBL were carried out. PCRs were used to detect resistance determinants in ESBL-EC for tetracycline, chloramphenicol, streptomycin and sulphonamides. The overall resistance for streptomycin and sulphamethoxazole was 100%, however the resistance rates for amoxicillin, tetracycline, chloramphenicol, amoxicillin/clavulante, trimethoprim, sulphamethoxazole/trimethoprim, gentamicin, amikacin, ciprofloxacin, cefotaxime, ceftazidime, aztreonam, cefepime and nitrofurantoin were 96%, 85%, 83%, 71%, 70%, 62%, 42%, 29%, 25%, 22%, 21%, 20%, 17% and 5% respectively. The whole collection was susceptible to fosfomycin, imipenem, tigecycline, cefoxitin, and colistin. Of 250 isolates, 21% were positive for ESBL. *bla*$_{\text{CTX-M-15-like}}$ was detected in all ESBL-EC. Among ESBL-EC, the prevalence of *tet(B), tet(A), tet(A), catI*, *cmlA, strA, strB, aadA, sul1*, *sul2,and sul3* was 89.9%, 8.2%, 2%, 88.9%, 51.11 %, 100%, 100%, 88.46%, 78.84%, 92.31%, 21.15%, respectively. The *bla*$_{\text{CTX-M-15-}}$*like, bla*$_{\text{TEM-}}$*like catI*, *tet(B)*, *sul2, strA*, *strB,* and *aadA* genes were the most prevalent resistance determinants. This study provides baseline data regarding the molecular bases of antimicrobial resistance in ESBL-EC from Riyadh.

Key words: *Escherichia coli*, ESBL, Antimicrobial resistances.

Urinary tract infection (UTI) is one of the commonest infectious disease presentations in medical practice. The most common cause of UTI in both community and health care settings is *Escherichia coli* (Auer *et al*., 2010). The choice of antibiotic for the treatment of UTI is limited by the rising rates of antibiotic resistance. The production of β-lactamases is the foremost mechanism of antibiotic resistance leading to treatment failure. Extended-spectrum β-lactamases (ESBLs), which hydrolyze extended-spectrum cephalosporins and are inhibited by β-lactamase inhibitors such as clavulanic acid, are spreading among *E. coli* (Al-agamy *et al*., 2014). Multi-drug resistance to unrelated antimicrobial classes such as aminoglycosides, chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, and fluoroquinolones is common among uropathogenic *E. coli*, leaving few therapeutic choices (Singh and Singh *et al*., 2014). The resistance to some antibiotics varies widely from one geographic location to another and also over time (Lagacé-Wiens *et al*., 2013; Al-Tawfiq and Anani, 2009; Karlowsky *et al*., 2006). The aims of this study were therefore to provide information

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regarding resistance patterns of 250 uropathogenic *E. coli* strains isolated in Riyadh, Saudi Arabia, to determine the prevalence of ESBL and to investigate the molecular resistance mechanisms to chloramphenicol, streptomycin, sulphonamide and tetracycline in ESBL-producing strains. Prevalence of the resistance genes; *sul1, sul2,sul3, tet(A), tet(B), tet(C), tet(D), tet(E), tet(G), catI, catII, catIII, cmlA, strA-strB* and *aadA* was investigated.

MATERIALS AND METHODS

Bacterial strains

A total of 250 non-duplicate *E. coli* strains were recovered from 465 mid stream urine samples from inpatients suffering from urinary tract infections (UTIs) in the Urology Department of a Hospital in Riyadh, Saudi Arabia. The isolates were collected from January to June 2012. Urine samples were inoculated onto MacConkey agar and incubated at 35°C overnight. After the incubation period, any lactose-fermenting colonies were picked and identified through a battery of biochemical tests, including indole, methyl red, Voges-Proskauer, citrate utilization, urease, and motility tests. *E. coli* confirmation was performed using API-20E (bioMerieux, Inc.). The identified isolates were stored at - 70^{0}C in tryptone soy broth containing 30% glycerol.

Antimicrobial susceptibility testing

MIC of 24 different antibiotics was determined by using E test strip (AB Biodisk, Solana, Sweden). MICs were performed and interpreted according to the guidelines of CLSI (CLSI, 2013). *E. coli* ATCC 25922 was used as a control strain.

Phenotypic detection of ESBL

ESBL production was detected using the 2013 CLSI recommendations for ESBL screening and confirmation tests (CLSI, 2013). The combination disc synergy test and ESBL strip E-tests (bioMerieux, Marcy L'Etoile, France) were performed for the detection and confirmation of ESBLs, respectively. For the combination disc synergy test, two disks of ceftazidime (30µg) were used, each with and without clavulanate (10µg). The isolate is ESBL producer if the zone expansion diameter is ≥ 5 mm larger with clavulanate than without. For the E-test, an ESBL strip containing ceftazidime and ceftazidime–clavulanate was used to determine the MIC ratio according to the manufacturer's instructions. *E. coli* ATCC 25922 (negative control) and *K. pneumoniae* ATCC 700603 (positive control) were used as reference strains.

Identifications of antibiotic resistance genes

The primers used in this study are listed in Table 1. Total bacterial DNA was prepared via the whole-cell boiled lysate procedure. PCR amplification was used to identify genes responsible for the resistance to: extended-spectrum cephalosporins, tetracycline, chloramphenicol, streptomycin, and sulphonamide. In total, ten different PCR protocols were applied and 22 antibiotic resistance genes were investigated. PCR was conducted to determine the gene responsible for the ESBL phenotype in the ESBL-EC. The amplification of bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M\text{-}1,}$ $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$, $bla_{\text{CTX-M-9}}$, and $bla_{\text{CTX-M-25}}$ genes was assessed via multiplex PCR using the primers and conditions described previously (Dallenne *et al*., 2010). ESBL-EC isolates (n=52) investigated in this study have been screened for the presence of tetracycline, chloramphenicol, sulphamethoxazole, and streptomycin resistance determinants. The The resistance genes; *tet*(A),-(B),-(C),-(D),-(E), -(G) (Ng *et al*., 2001) for tetracycline resistance, *catI*, *catII*, *catIII* (Vassort-Bruneau *et al*., 1996), *cmlA* (Keyes *et al*., 2000) for chloramphenicol resistance, *sul1*, *sul2*, *sul3 (*Wu *et al*., 2010; Kerrn *et al*., 2002) for sulfonamide resistance, *strA*, *strB* (Tamang *et al*., 2007) and *aad* (Madsen *et al*., 2000) for streptomycin resistance. Positive and negative controls were included in all PCR assays. All PCR assays were done in Techne thermocycler (Techne, UK). PCR products were separated electrophoretically in a 1-2% agarose gel using 1xTBE, visualized by staining with 0.5 µg/ml ethidium bromide and examined in UV light and photographed using video documentation system.

RESULTS

Antimicrobial susceptibility

MICs of antimicrobial agents were determined for 250 uropathogenic *E. coli* isolates. The results of MICs are shown in Table 2. No resistance was recorded in this study to cefoxitin,

Table 1. Primers used in the study

PCR	Genes	Primer	Sequence (5'—3')	Amplicons	Reference
Multiplex TEM, SHV	TEM variants	MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	800	Dallenne et al., 2010
		MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC		
	SHV variants	MultiTSO-S_for	AGCCGCTTGAGCAAATTAAAC	713	
		MultiTSO-S_rev	ATCCCGCAGATAAATCACCAC		
Multiplex CTX-M group 1, group 2 and group 9	CTX-M group 1	MultiCTXMGp1_for	TTAGGAARTGTGCCGCTGYA	688	
		MultiCTXMGp1-2_rev	CGATATCGTTGGTGGTRCCAT		
	CTX-M group 2	MultiCTXMGp2_for	CGTTAACGGCACGATGAC	404	
		MultiCTXMGp1-2_rev	CGATATCGTTGGTGGTRCCAT		
	CTX-M group 9	MultiCTXMGp9_for	TCAAGCCTGCCGATCTGGT	561	
		MultiCTXMGp9_rev	TGATTCTCGCCGCTGAAG		
CTX-M group 8/25	CTX-M-8, CTX-M-25	CTX-Mg8/25_for	AACRCRCAGACGCTCTAC	326	
		CTX-Mg8/25_rev	TCGAGCCGGAASGTGTYAT		
Muliplex Sul1, sul2 and sul3	*Sul1*	Sul1-F	CGGCGTGGGCTACCTGAACG	433	Kerrn *et al.* 2002
		Sul 1- B	GCCGATCGCGTGAAGTTCCG		
	Sul2	Sul 2-F	GCGCTCAAGGCAGATGGCATT	293	Kerrn *et al.*2002
		Sul 2-B	GCGTTTGATACCGGCACCCGT		
	Sul3	Sul3-F	CAGATAAGGCAATTGAGCATGCTCTGC	569	Wu et al., 2010
		Sul3-B	AGAATGATTTCCGTGACACTGCAATCATT		
aadA-PCR	*aadA*	aad-F	GTG GAT GGC GGC CTG AAG CC	525	Madsen et al. 2000
		aad-R	AAT GCC CAG TCG GCA GCG		
StrA-strB-PCR	*strA-strB*	Str-A-F	ATG GTG GAC CCT AAA ACT CT	893	Tamang et al.2007
		Str-B-R	CGT CTA GGA TCG AGA CAA AG		
Multilex *tet* genes	*tet*(A)	tetA-F	GCT ACA TCC TGC TTG CCT TC	210	Ng et al., 2001
		tetA-R	CAT AGA TCG CCG TGA AGA GG		
	tet(B)	tetB-F	TTG GTT AGG GGC AAG TTT TG	659	
		tetB-R	GTA ATG GGC CAA TAA CAC CG		
	tet(C)	tetC-F	CTT GAG AGC CTT CAA CCC AG	418	
		tetC-R	ATG GTC GTC ATC TAC CTG CC		
	tet(D)	tetD-F	AAA CCA TTA CGG CAT TCT GC	787	
		tetD-R	GAC CGG ATA CAC CAT CCA TC		
	tet(E)	tetE-F	AAA CCA CAT CCT CCA TAC GC	278	
		tetE-R	AAA TAG GCC ACA ACC GTC AG		
	tet(G)	tetG-F	GCT CGG TGG TAT CTC TGC TC	468	
		tetG-R	AGC AAC AGA ATC GGG AAC AC		
cmlA-PCR	*cmlA*	cmlA-F	CCG CCA CGG TGT TGT TGT TAT C	698	Keyes et al.
		cmlA-R	CAC CTT GCC TGC CCA TCA TTA G		
Multiplex-cat-PCR	catI	catI-F	GGCATTTCAGTCAGTTG	585	Vassort-Bruneau et al., 1996
		catI-R	CCGCCCTGCCACTCATC		
	catII	catII-F	CCTGGAACCGCAGAGAAC	495	
		catII-R	CCTGCTGAAACTTTGCCA		
	catIII	catIII-F	ATTGGCTTCGCCGTGAGC	508	
		catIII-R	AGTCTATCCCCTTCTTG		

Table 2. Antimicrobial resistance patterns and MICs of 250 *E. coli* strains

Antimicrobial agents	Resistance pattern of uropathogenic *E. coli* isolates											
	Total isolates (n=250)				ESBL-EC (n=52)				Non-ESBL-EC (n=198)			
	Susceptible		Resistant		Susceptible		Resistant		Susceptible		Resistant	
	No	%	No	%	No	%	No	%	No	%	No	%
Amikacin	177	70.8	73	29.2	29	55.8	23	44.2	148	74.7	50	25.3
Amoxicillin	10	4.0	240	96.0	0	0.0	10	100.0	10	100.0	0	0.0
Amoxicillin/ clavulanate	72	28.8	178	71.2	7	13.5	45	86.5	65	32.8	133	67.2
Aztreonam	200	80.0	50	20.0	2	3.85	50	96.15	198	100.0	0	0.0
Cefepime	208	83.2	42	16.8	10	19.2	42	80.8	198	100.0	0	0.0
Cefotaxime	195	78.0	55	22.0	0	0.0	52	100.0	195	98.5	3	1.5
Cefotaxime/ clavulanate	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0	0	0.0
Cefoxitin	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0	0	0.0
Ceftazidime	198	79.2	52	20.8	0	0.0	52	100.0	198	100.0	0	0.0
Chloramphenicol	42	16.8	208	83.2	7	13.5	45	86.5	35	17.7	163	82.3
Ciprofloxacin	187	74.8	63	25.2	25	48.1	27	51.9	162	81.8	36	18.2
Doxycycline	41	16.4	209	83.6	3	5.8	49	94.2	38	19.2	160	80.8
Fosfomycin	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0	0	0.0
Gentamicin	146	58.4	104	41.6	21	40.4	31	59.6	125	63.1	73	36.9
Imipenem	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0	0	0.0
Minocycline	50	20.0	200	80.0	4	7.7	48	92.3	46	23.2	152	76.8
Nitrofurantoin	237	94.8	13	5.2	49	49.2	3	5.8	188	94.95	10	5.05
Streptomycin	0	0.0	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0
Sulfamethoxazole	0	0.0	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0
Sulfamethoxazole/ Trimethoprim	95	38.0	155	62.0	6	11.5	46	88.5	89	44.95	109	55.05
Tetracycline	38	15.2	212	84.8	3	5.8	49	94.2	35	17.7	163	82.3
Tigecycline	250	100.0	0	0.0	52	100.0	0	0.0	198	100.0	0	0.0
Trimethoprim	75	30.0	175	70.0	11	21.15	41	78.85	64	32.3	134	67.7

Table 3. MIC of extended-spectrum β-lactamase-positive *E. coli* and their antibiotic resistance determinants

ESBL-EC isolates	MIC (µg/ml)									Antibiotic Resistance genes														
	CTX	CAZ	ATM	CPM	CIP	TET	CHL	STR	SXT	TEM	SHV	CTX-M-15	*tetA*	*tetB*	*tetC*	*cat1*	*cmlA*	*strA*	*strB*	*aad*	*sul1*	*sul2*	*sul3*
ESBL-EC1	>256	192	96	48	0.5	>256	>128	>256	256	+	-	+	+	+	-	+	-	+	+	+	+	+	-
ESBL-EC2	>256	>256	>256	>256	>32	128	>128	256	256	+	+	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC3	128	96	48	6	0.5	64	>128	256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	+
ESBL-EC4	>256	>256	128	96	16	192	>128	256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC5	>256	>256	>256	128	24	>256	>128	256	256	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC6	>256	>256	>256	32	0.25	>256	>128	256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC7	>256	>256	>256	32	8	48	>128	>256	>512	+	+	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC8	>256	>256	>256	16	0.5	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC9	>256	>256	>256	>256	>32	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC10	>256	>256	>256	>256	1	128	4	128	>512	+	-	+	-	+	-	-	-	+	+	-	+	-	+
ESBL-EC11	>256	>256	>256	>256	0.75	192	>128	128	128	+	-	+	-	+	-	+	+	+	+	-	-	+	-
ESBL-EC12	192	128	192	4	>32	96	>128	32	>512	+	-	+	-	+	-	+	-	+	+	-	+	+	-
ESBL-EC13	>256	>256	>256	192	0.25	64	4	>256	>512	+	-	+	-	+	-	-	-	+	+	+	-	+	+
ESBL-EC14	128	48	1.5	128	0.5	1	2	128	192	+	-	+	-	-	-	-	-	+	+	-	+	-	+
ESBL-EC15	>256	>256	32	48	>32	>256	>128	256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC16	>256	>256	64	64	0.5	128	32	256	>512	+	-	+	-	+	-	-	+	+	+	+	+	+	-
ESBL-EC17	>256	>256	48	64	>32	48	2	256	128	+	-	+	-	-	+	-	-	+	+	+	-	+	-
ESBL-EC18	>256	>256	>256	24	0.5	24	32	256	>512	+	-	+	+	-	-	-	+	+	+	+	+	+	-
ESBL-EC19	>256	>256	24	16	16	2	3	256	256	+	-	+	-	-	-	-	-	+	+	+	-	-	+
ESBL-EC20	>256	>256	192	12	0.25	>256	>128	256	>512	+	+	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC21	>256	>256	>256	>256	24	>256	>128	256	>512	+	-	+	-	+	-	+	+	+	+	+	-	+	+
ESBL-EC22	192	>256	>256	>256	>32	128	24	128	>512	+	-	+	-	+	-	-	+	+	+	-	+	+	-
ESBL-EC23	>256	>256	>256	96	0.12	192	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC24	>256	>256	>256	3	16	96	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC25	>256	>256	>256	2	0.06	64	16	256	>512	+	+	+	+	-	-	-	+	+	+	+	+	+	-
ESBL-EC26	>256	>256	>256	>256	>32	>256	>128	256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC27	>256	>256	>256	32	0.12	>256	>128	256	128	+	-	+	-	+	-	+	+	+	+	+	-	+	-
ESBL-EC28	>256	>256	>256	192	>32	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC29	>256	>256	128	128	16	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-

ESBL-EC30	>256	>256	16	128	12	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC31	>256	>256	192	6	0.06	96	>128	256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC32	>256	>256	1	2	0.25	32	>128	256	>512	+	-	+	+	-	-	+	+	+	+	+	-	+	+
ESBL-EC33	>256	>256	>256	>256	0.5	>256	>128	256	256	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC34	>256	>256	>256	128	0.25	>256	>128	256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC35	>256	>256	128	48	>32	>256	>128	256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC36	>256	>256	>256	12	0.25	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC37	>256	>256	>256	24	24	>256	>128	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC38	>256	>256	>256	32	0.12	128	>128	>256	>512	-	+	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC39	>256	>256	>256	32	>32	1	3	128	256	+	-	+	-	-	-	-	-	+	+	-	-	+	+
ESBL-EC40	>256	>256	>256	32	0.25	>256	64	64	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC41	128	>256	6	2	0.5	16	64	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	-
ESBL-EC42	>256	>256	>256	>256	1	>256	48	>256	>512	+	-	+	-	+	-	+	-	+	+	+	+	+	+
ESBL-EC43	>256	>256	>256	24	>32	>256	>128	>256	256	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC44	>256	>256	96	>256	2	>256	>128	>256	128	+	-	+	-	+	-	+	+	+	+	+	-	+	-
ESBL-EC45	>256	>256	>256	0.5	0.25	>256	>128	>256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC46	>256	>256	192	48	16	24	>128	>256	>512	+	-	+	-	-	-	+	+	+	+	+	-	+	+
ESBL-EC47	>256	>256	>256	192	24	128	>128	>256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC48	>256	>256	>256	128	0.06	>256	>128	>256	>512	+	-	+	-	+	-	+	+	+	+	+	+	-	+
ESBL-EC49	>256	>256	32	16	>32	>256	96	>256	>512	+	-	+	-	+	-	+	+	+	+	+	+	+	-
ESBL-EC50	>256	>256	64	3	24	>256	1	128	>512	+	-	+	-	+	-	-	-	+	+	+	+	+	-
ESBL-EC51	96	128	64	32	6	>256	>128	>256	256	+	+	+	-	+	-	+	+	+	+	+	-	+	+
ESBL-EC52	192	128	8	0.5	0.12	>256	48	>256	>512	+	-	+	-	+	-	-	+	+	+	+	+	+	-

CTX= Cefotaxime, CAZ = ceftazidime, CPM = cefepime, CIP= ciprofloxacin, TET= tetracycline, CHL = chloramphenicol, STR = streptomycin, SXT= sulfamethoxazole

colistin, fosfomycin, imipenem, and tigecycline. Of the examined *E. coli* isolates, 96% (240/250) were resistant to amoxicillin, whereas 71.2% (178/250) were resistant to amoxicillin/clavulanic acid. The percentage of resistance to extended-spectrum cephalosporins: cefotaxime, ceftazidime, aztreonam and cefepime were 22%, 21%, 20% and 17% respectively. Resistance to gentamicin, amikacin and ciprofloxacin was exhibited by 42%, 29% and 25% of the tested *E. coli*, respectively. All of the isolates were completely resistant to aminoglycoside streptomycin. 85% of the isolates were resistant to tetracycline; 83% were resistant to doxycycline; and 80% were resistant to minocycline. Furthermore, the uropathogenic *E. coli* isolates showed the following levels of resistance to antifolate antibacterial: 100% of the isolates were resistant to sulfamethoxazole, and 70% were resistant to trimethoprim, whereas 62% of the isolates showed resistance to a combination of sulfamethoxazole/trimethoprim. Chloramphenicol resistance was observed in 83% of the *E. coli* isolates. Nitrofurantoin, which is the most commonly used drug for the treatment of uropathogenic *E. coli*, 5% of the isolates exhibited resistance. The antimicrobial resistance rates were higher for ESBL-EC than non-ESBL-EC isolates (Table 2). The MIC of ESBL-EC is illustrated in Table 3.

Prevalence of ESBL and PCR characterization

ESBL production was determined by combination disk test and E test strip method. According to the phenotypic detection, the prevalence of ESBL-EC was 20.8% (52/250). The PCR experiments revealed that the prevalence of $bla_{\text{CTX-M-15-}}$like, $bla_{\text{TEM-}}$like and $bla_{\text{SHV-}}$like in ESBL-EC was 100% (52/52), 98.07% (51/52), and 11.53% (6/52).

Prevalence of the resistance determinants

Fifty two ESBL-EC strains were chosen for molecular characterization of resistance genes. The results of this characterization are summarized in Table 3. Forty nine (94.23%) out of 52 ESBL-EC were resistant to tetracycline and *tet* genes were detected in all of the resistant ESBL-EC. The presence of six different tetracycline resistance genes was investigated through multiplex PCR. Among the tested *tet* genes, *tet(B)*, *tet(A)* and *tet(C)* were detected. *tet(B)* gene was the most prevalent of these genes being found in 89.8% (44/49) of the tested isolates. The prevalence of *tet(A)* and *tet(C)* were 8.16% (4/49) and 2.04% (1/49) respectively. Forty five (86.54%) out of 52 ESBL-EC were resistant to chloramphenicol. Four different chloramphenicol resistance genes, *catI*, *catII*, *catIII* and *cmlA* genes were checked by PCR. Among the tested chloramphenicol resistance genes, *catI* and *cmlA* were detected with prevalence of 88.89% (n=40/45) and 51.11% (n=23/45) respectively. The *catI* and *cmlA* genes were detected in combination in 20/45 (43.18%). The resistance rate to streptomycin and sulphamethoxazole was 100% (52/52). The *strA+strB* genes were detected in the whole streptomycin resistant ESBL-EC while *aadA* was detected in 46/52 (88.46%). Multiplex PCR were sought to detect three different *sul* genes. The *sul* genes were detected in 100% of the investigated ESBL-EC strains. The prevalences of *sul1*, *sul2* and *sul3* were 78.84% (41/52), 92.31% (48/52) and 21.15% (11/52), respectively. *sul1* and *sul2* were found in combination in 36/52 (69.32%) of ESBL-EC. *sul2* was concomitant with *sul3* in six isolates (11.54%). *sul1* was concomitant with *sul3* in three ESBL EC (5.77%). All three *sul* genes were found in two isolates (2.35%). *sul2* and *sul3* were found alone in four and one ESBL-EC isolates, respectively. However *sul1* was always found concomitant with *sul2* gene.

DISCUSSION

UTI is a common disease in the community, and a matter of concern due to the increasing resistance of microorganisms to first line antibiotics and the emergence of multiresistant strains producing ESBL in the community (Leal *et al*., 2013). The available data on antimicrobial resistance and the mechanisms underlying this resistance are limited, especially in most developing countries (Chuanchuen and Padungtod, 2009). Nitrofurantoin and ciprofloxacin are widely used to treat UTI, and resistance to these drugs was found to be lower (5% and 25%, respectively) in the present study than reported in a previous study (67.5% and 46.7%, respectively) (Al-Tawfiq and Anani, 2009). Amikacin showed considerable activity against the isolates, as only 29% of the tested *E. coli* isolates were resistant, whereas 42% of the isolates were resistant to gentamicin. Sulfonamide and trimethoprim were not effective

in the treatment of the uropathogenic *E. coli*. The resistance rates to sulfamethoxazole and trimethoprim were 100% and 70%, respectively, although resistance decreased to 62% when sulfamethoxazole was combined with trimethoprim. This finding is in agreement with a previous study that examined the antimicrobial susceptibility patterns of *E. coli* causing UTIs in Saudi Arabia (Al-Tawfiq and Anani, 2009), in which the authors reported that 57.8% of the examined uropathogenic *E. coli* isolates from nosocomial infections were resistant to sulfamethoxazole/trimethoprim.

The rates of resistance to aztreonam, cefotaxime, and ceftazidime were 20, 21, 22% respectively in this study ranged All isolates were susceptible to cefotaxime, and ceftazidime when combined with clavulanic acid. This result indicates that the resistance to extended spectrum cephalosporins was due to ESBLs and suggests that the prevalence of ESBL-EC is increasing in Saudi Arabia. In the present study, the results revealed that 20.8% of 250 *E. coli* isolates produced ESBL. The prevalence of ESBLs among enterobacterial clinical isolates has increased significantly over the past two decades (Peirano and Pitout, 2010). Regardless of this increase in ESBLs worldwide, there is a rarity of local reports on the prevalence of ESBL positive *E. coli* (Al-Otaibi and Bukhari, 2013, Marie *et al*., 2013, Hassan and Abdalhamid, 2014, Al agamy *et al*., 2014). In the recent previous studies in Saudi Arabia, the prevalence of ESBLs positive *E. coli* was 20.4% (Al-Agamy *et al*., 2014), 33.3% (Al-Otaibi and Bukhari, 2013), 35.8% (Hassan and Abdalhamid, 2014). In the present study, the results revealed that 52 (20.8%) of 250 *E. coli* isolates produced ESBL. On other hand the early previous local studies were found that the prevalence rates of ESBL-EC were 6.5% and 10.3% in 2002 and 2004, respectively (Kader and Kumar, 2005), and were 15.7% and 4.8% from inpatients and outpatients, respectively (Khanfar *et al*., 2009). After determination of ESBL by phenotypic method, PCR was used to determine the genotypic of ESBL. In the present study, CTX-M-type was the most prevalent ESBL in ESBL-EC isolates followed by TEM and SHV. The prevalence rate of CTX-M-15-like, TEM, and SHV was 100% (52/52), 98.07% (51/52), and 11.53% (6/52) respectively. This finding is in agreement with a previous study in Saudi Arabia and worldwide that stated that CTX-M-15-like is a dominate ESBL in ESBL-EC (Al-Agamy *et al*., 2014; Hassan and Abdalhamid, 2014; D'Andrea *et al*., 2013; Shibl *et al*., 2012).

Antibiotic resistant determinants for tetracycline, chloramphenicol, streptomycin, and sulfonamide were amplified in 52 ESBL-EC isolates. Tetracycline-specific efflux pump proteins are the leading causes of tetracycline resistance in *E. coli* (Nguyen *et al*., 2014; Mullany *et al*., 2012; Mirzaagha *et al*., 2011 Roberts, 1996). The *tet* efflux genes *tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E) and *tet*(G) have previously been identified (Mirzaagha *et al*., 2011; Wilkerson *et al*., 2004). Screening for resistance determinants showed that the majority of tetracycline-resistant isolates harboured the *tet*(B) efflux gene, followed less frequently by *tet*(A) and *tet*(C). These findings are consistent with other previous studies (Mirzaagha *et al*., 2011; Ahmed *et al*., 2010; Mirzaagha *et al*., 2009; Walk *et al*., 2007; Wilkerson *et al*., 2004; Blake *et al*., 2003) who reported that most tetracycline-resistant isolates possessed *tet*(B) followed by *tet*(A) and *tet*(C) determinants. The *tet*(B) gene exhibits a wide host range because it resides in highly mobile genetic elements that readily undergo transfer between bacterial genera (Mirzaagha *et al*., 2011; Mirzaagha *et al*., 2009; Blake *et al*., 2003).

In the developed world, chloramphenicol is virtually irrelevant clinically and has been banned in the U.S. and other countries for use in humans or food animals due to its potential toxic effects on humans. In most of the developing world, its use is also limited by high levels of resistance likely due to the low-cost of the antimicrobial and unregulated, widespread over use (Frye and Jackson, 2013). In the current study, an enzymatic chloramphenicol resistance gene was more prevalent than non-enzymatic resistance gene. As shown in Table (3) enzymatic chloramphenicol resistance gene, *catI*, was more tied with high chloramphenicol resistance phenotype (MIC e"96 mg/ml), whereas non-enzymatic chloramphenicol resistance gene, *cmlA*, might link to intermediate resistance to chloramphenicol (MIC £48 mg/ml). The present results are in agreement with many reports that enzymatic mechanism is more common than non-enzymatic mechanisms (Ahmed *et al*., 2010; Al-Agamy, 2006). Among the genes involved in the enzymatic inactivation mechanism, only *catI*

was identified with prevalence of 88.89%. The non-enzymatic efflux mechanism, *cmlA*, plays an important role in the chloramphenicol resistance with prevalence of 51.11%. The *cmlA* gene was alone in five out of 45 chloramphenicol resistant ESBL-EC isolates which showed chloramphenicol MICs of d"48µg/ml. However, *cmlA* accompanied *catI* in 18 ESBL-EC isolates that displayed chloramphenicol MICs of ³ 128 µg/ml. Chloramphenicol acetylating gene, *catI*, was detected alone in 48.88% of chloramphenicol resistant ESBL-EC isolates.

Sulfonamides are some of the most frequently used antimicrobials for treating uncomplicated UTI, and they are widely administered in Saudi Arabia (Al-Tawfiq and Anani, 2009). The rate of resistance to sulfamethoxazole was observed to be 100% in the present study, thus suggesting that sulfonamide is not effective in the treatment of uropathogenic *E. coli*. Interestingly, the prevalence of sulfonamide resistance among *uropathogenic E. coli* remains high in the UK, despite an almost complete withdrawal of the antibiotic from human medical use in that country (Enne *et al*., 2001; Kerrn *et al*., 2002). Three *sul* genes (*sul1, sul2* and *sul3*) that confer resistance to sulfonamides have been identified to date. *sul2* (92.31%; 48/52) was the most prevalent *sul* gene identified in the present study, followed by *sul1* (8.84%; 41/52) then *sul3* (21.15% (11/52), in accordance with previous observations (Wu *et al*., 2010; Trobos *et al*., 2008; Hammerum *et al*., 2006; Kerrn *et al*., 2002).

Despite the fact that the use of streptomycin in Saudi Arabia has been restricted to the treatment of *Mycobacterium tuberculosis* and enteric Gram-negative bacteria, in addition to veterinary medicine, this study found that the level of streptomycin resistance was 100% among the examined *E. coli* isolates. Three aminoglycoside-modifying enzyme genes, *aadA*, *strA* and *strB*, were investigated via PCR. *strA* and *strB* conferred resistance to streptomycin, whereas *aadA* conferred resistance to streptomycin and spectinomycin. The high prevalences of the *strA and strB* genes (100%) as well as the *aadA* gene (88.46%) detected in the *ESBL-EC* isolates suggests that these genes play a major role in conferring resistance to streptomycin in *E. coli*. The *aadA+strA+strB* genes were detected in 88.46% of the investigated isolates, whereas 11.54% of isolates contained only *strA+strB*. Kikuvi *et al*. (2007) also detected isolates containing only the *strA+strB* genes (24%), whereas the majority of the isolates they examined (72.4%) contained all three genes (*strA+strB+aadA1*). Thus, the current study is in accordance with the findings of Kikuvi *et al*. (2007), who determined that the prevalence of *strA+strB* was higher than that of *aadA* and that *strA* was always accompanied by *strB* (Kikuvi *et al*., 2007), similar to the present results. Isolates harboring the *strA, strB* and *aadA* genes showed higher streptomycin MICs than those harboring *strA* and *strB* alone.

CONCLUSION

To my knowledge this is the first study to genetically characterize resistance to tetracycline, chloramphenicol, streptomycin and sulphamethoxazole in ESBL-EC from Riyadh, Saudi Arabia. The whole collection of isolates was sensitive to cefoxitin, colistin, fosfomycin, imipenem, and tigecycline. Nitrofurantoin is still effective against uropathogenic *E.coli*. This study provides useful information regarding the dissemination of antimicrobial resistance genes among uropathogenic *E. coli* isolates. Prevalence of ESBL-EC was intermediately high in Riyadh. CTX-M-15-like gene is the dominant ESBL genes in ESBL-EC. CTX-M-15-like, *tet(B), sul2, cat1* and *strA* and *strB* were found to be the most prevalent resistance genes, encoding extended-spectrum cephalosporin, tetracycline, sulphamethoxazole, chloramphenicol and streptomycin resistance, respectively.

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